

## HERBIVORE ASSOCIATED MICROORGANISMS MODULATING INDUCED PLANT DEFENSES

F. MIRIAM AMALA SALEESHA<sup>1</sup>, V. R. SAMINATHAN<sup>2</sup>  
S. SHEEBA JOYCE ROSELEEN<sup>1</sup> & V. RAJAN BABU<sup>3</sup>

<sup>1</sup>Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute  
Navalur, Kuttapattu, Tiruchirappalli, Tamil Nadu, India

<sup>2</sup>Department of Plant Protection, Horticultural College and Research Institute (Women)  
Navalur, Kuttapattu, Tiruchirappalli, Tamil Nadu, India

<sup>3</sup>Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College and Research Institute  
Navalur, Kuttapattu, Tiruchirappalli, Tamil Nadu, India

### ABSTRACT

*Animals that feed on plant tissues or plant products are called herbivores. Plants present various nutritional and defensive barriers against herbivores; nevertheless, insects have evolved a diverse array of mechanisms that enable them to feed and develop on live plant tissues. (Hanson and Moran, 2013). Induced plant defenses in response to herbivore attack are modulated by cross-talk between jasmonic acid JA and salicylic acid SA signaling pathways. Oral secretions from some insect herbivores contain effectors that overcome these antiherbivore defenses (Chung et al., 2013). Bacteria mediating the plant response to herbivore attack can be allocated to three major groups, based on their location onto the herbivore: environmental (or external), digestive system (internal extracellular) and endosymbionts (internal intracellular) (Schausberger, 2018). Herbivores possess diverse microbes in their digestive systems, affects the host physiology and these microbial symbionts can modify plantinsect interactions (Su et al., 2015). Herbivore associated bacteria not only influence the immune phenotype of their insect host, but also the immune phenotype of the insect's host plant (Wang et al., 2017).*

**KEYWORDS:** *Herbivores, Jasmonic Acid, Salicylic Acid, Symbionts & Immune Phenotype*

**Received:** Mar 19, 2019; **Accepted:** Apr 10, 2019; **Published:** May 06, 2019; **Paper Id.:** IJASRJUN201923

### INTRODUCTION

Plants and insects have been living together for more than 350 million years. In co-evolution, both have evolved strategies to avoid each other's defense systems. This evolutionary arms race between plants and insects has resulted in the development of an elegant defense system in plants that has the ability to recognize the non-self molecules or signals from damaged cells, much like the animals, and activates the plant immune response against the herbivores (Howe and Jander, 2008). To counter the herbivore attack, plants produce specialized morphological structures or secondary metabolites and proteins that have toxic, repellent and anti-nutritional effects on the herbivores (Usharani and Jyothsna, 2010).

## PLANT DEFENSE AGAINST HERBIVORES

Plants confront the herbivores both directly by affecting host plant preference or survival and reproductive success (direct defense), and indirectly through other species such as natural enemies of the insect pests (indirect defense) (Dudareva *et al.*, 2006). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores (Hanlet *et al.*, 2007). Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attracts natural enemies of the herbivores and by providing food (e.g., extrafloral nectar) and housing to enhance the effectiveness of the natural enemies (Arimura *et al.*, 2009).

### Jasmonic Acid

JA is the most important phytohormone, activates the expression of both direct and indirect plant defenses against herbivores (Usharani and Jyothsna, 2010). Chewing of plant parts by insects causes the dioxygenation of linoleic acid and linolenic acid. Defensive responses induced by JA include antioxidative enzymes, PIs, VOCs, alkaloid production, trichome formation and secretion of EFN (War *et al.*, 2012).

### Salicylic Acid

Salicylic acid (SA), a benzoic acid derivative, is an important phytohormone involved in the regulation of plant defense (War *et al.*, 2011). It is an important endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in defense in addition to their impact on plant growth and development (Rivas-san Vicente and Plasencia, 2011). Responses to SA depend on a regulatory protein called Non-Expressor of Pathogenesis-Related Genes1 (NPR1) (Pieterse and Van-loon, 2004). The NPR1 gene is activated through redox pathways by SA accumulation and is translocated to the nucleus, however, it does not bind to DNA directly, but acts through transcription factors. SA induces greater defense against piercing and sucking type of insect pests than the chewing ones (Zhao *et al.*, 2009).

### Polyphenol Oxidase (PPO)

The PPOs are important enzymes in plants that regulate feeding, growth, and development of insect pests and play a leading role in plant defense against the biotic and abiotic stresses (Bhonwong *et al.*, 2009). PPOs can function in the following ways:

- PPO-generated quinones could alkylate essential amino acids, decreasing plant nutritional quality,
- Quinones produce oxidative stress in the gut lumen through redox cycling, and
- Quinones and ROS produced by phenolic oxidation have toxic effects on herbivores (War *et al.*, 2012).

## MICROBIAL INTERACTIONS

The theory on insect-plant interactions cannot progress realistically without consideration of the third trophic level (Price *et al.*, 1980). In their discussion of the third trophic level, it includes parasitoids, predators and microbes. The microbes may mediate the interactions of insect herbivores with their host and the novel interactions among microbes, plants, and herbivores are,

### **Insect-Associated Microbes**

Insect-associated microbes can suppress plant defenses and detoxify defensive phytochemicals that enhance insect fitness.

### **Polydnaviruses**

Symbiotic polydnaviruses of some parasitoids that are released into the host during parasitization can modify the expression of herbivore-associated molecular patterns (HAMPs) and effectors, thereby suppressing plant defenses to promote host growth and parasitoid fitness.

### **Phytopathogens**

Plant pathogens can cause diseases to plants.

### **Plant-Beneficial Microbes**

Plant-beneficial microbes can influence insect behavior and fitness through changes in plant biomass, nutritional quality, defensive properties and attraction of natural enemies.

### **Entomopathogens**

Entomopathogens may potentially influence plant defenses directly as endophytes or indirectly by modifying the composition of insect oral secretions. (Shikano *et al.*, 2017)

## **INSECT-MICROBE SYMBIOTIC ASSOCIATION**

Resident microorganisms in insects act as “biochemical brokers” that enable insects to exploit plants. The chemical functions mediated by these microorganisms could include detoxification of plant allelochemicals, degradation of plant cell walls, and biosynthesis of nutrients that are essential for the insect but in short supply in the plant food (Douglas, 2013). Symbionts, defined as microbes that form persistent, non-invasive associations with hosts, are common in insects generally and in herbivorous insects in particular (Hansan and Moran, 2013).

### **Categories of Symbiont**

Microbes have persistent associations with their hosts and that do not cause overt pathogenic symptoms. Insect symbionts can be divided into three general categories based on their location relative to the insect body: environmental (or external), digestive system (internal extracellular) and endosymbionts (internal intracellular) (Schausberger, 2018). Few symbionts are **intracellular** or housed within the body cavity and are passed between generations through eggs, resulting in maternal inheritance. Heritable, usually intracellular, symbionts are found in a variety of insect groups, which often evolve specialized cells (bacteriocytes) for housing them. Second, microbes **inhabit the guts** of most animals including most insects, living within the gut lumen where they form communities of varying complexity in different insect species. Third, microbes regularly colonize food before it is ingested and may impact its composition. These **environmental symbionts** most likely are important in insects that feed on a stored food resource within a nest or localized feeding area such as a gall or gallery (Hansan and Moran, 2013).

### HAOs Directly Affect Plant Responses to Herbivory

To activate defense responses, plants recognize insect attack by their damage pattern by perceiving herbivore-derived chemical cues (herbivore-associated elicitors or herbivore-associated molecular patterns) (Bonaventure, 2012). The elicitors induce signal-transduction pathways regulated by phytohormones and gene transcripts that modulate herbivory-induced responses in plants (Erb *et al.*, 2012). When honeydew excreted by aphids drops onto the plant, it may suppress defense-related jasmonic acid accumulation by inducing salicylic acid, suggesting that bacteria within the honeydew may make plants less resistant to the aphids (Schwartzberg&Tumlinson,2014; Zhu *et al.*, 2014) .

### HAOs Indirectly Affect Plant Responses to Herbivory

#### HAOs Influence Host Behavior

The presence of HAOs leads to changes in the behavior of the host insect, including reproduction, feeding behavior and locomotion (Hughes *et al.*, 2012). Changes in movement of hosts as a result of parasitism have been well investigated in different parasite-host systems. For instance, fungal or viral infections may manipulate the behavior of their insect host such that the host now moves to the top of the canopy, which is beneficial for reproduction and spread of the parasites (Hoover *et al.*, 2011)

#### HAOs Influence Host Physiology

Microbial symbionts can provide essential nutrients to the host, such as amino acids, vitamins, and sterols. Symbionts of herbivorous insects greatly improve nutrient uptake and open niches to their insect host, allowing colonization of a broad range of host plants (Douglas, 2009). Endosymbionts (*Arsenophonus* and *Wolbachia* in *B. tabaci*) may also contribute to herbivore resistance to insecticides (Ghanim & Kontsedalov, 2009).

**Table 1: List of HAM Involves in Modulating Plant Defenses**

S. No	Insect Host	Microbes	Plant	Function	Reference
1.	<i>Leptinotarsa decemlineata</i>	<i>Stenotrophomonas</i> , <i>Pseudomonas</i> , <i>Enterobacter</i> (OS/REG)	Tomato	Decreased JA responsive defense and increased SA accumulation	Chug <i>et al.</i> , 2013
2.	<i>Diabrotica virgifera virgifera</i>	<i>Wolbachia</i> (OS/REG)	Maize	Supress defense related genes	Shikano <i>et al.</i> , 2017
3.	<i>Macrosiphum euphorbiae</i>	<i>Buchnera aphidicola</i>	Potato	Chaperonin GroEL – elicits pattern triggered immunity	Chaudhary <i>et al.</i> , 2014
4.	<i>Bactericera cockerelli</i>	<i>Liberibacter psyllaurous</i>	Tomato	Alters JA-and SA-regulated defenses	Casteel <i>et al.</i> , 2012
5.	<i>Helicoverpa zea</i>	<i>Enterobacter ludwigii</i> (OS/REG)	Tomato	Induce the expression of GOX elicits the production of JA regulated defenses	Wang <i>et al.</i> , 2017
6.	<i>Dentroctonus ponderosae</i>	<i>Serratia</i> , <i>Rahnella aquatilis</i>	Pine	Reduced concentration of monoterpenes	Boone <i>et al.</i> , 2013
7.	<i>Bemisia tabaci</i>	<i>Hamiltonella defensa</i>	Tomato	Suppression of JA-related genes	Su <i>et al.</i> , 2015

## HERBIVORE – MICROBE – PLANT INTERACTION

### Herbivore Associated-Microbe Indirectly Induces Defense by Triggering a Salivary Elicitor

Insect-associated bacteria influence the elicitation of defensive reactions in the host plants. *Helicoverpa zea* gut-associated bacteria indirectly mediate plant-insect interactions by triggering salivary elicitors (GOX). *Enterobacter ludwigii* was one of three bacterial isolates that increased GOX activities in the labial glands of *H.zea* larvae and suppressed PPO activities in tomato plants. *Enterobacter ludwigii*-inoculated caterpillars induce JA-responsive defenses in tomato plants. To investigate whether gut-associated bacteria affect defense-related JA and SA signaling pathways, the expression of selected defense-related genes in tomato plants damaged by *H.zea* larvae inoculated with *E.ludwigii* was measured. Tomato plants damaged by *E.ludwigii*-inoculated *H.zea* larvae had significantly higher expression levels of the JA-responsive genes *Pin2*, *CysPI* and *PPOF* compared with plants damaged by  $MgCl_2$  solution-inoculated caterpillars, while the expression level of SA responsive *Pr1a* (P4) was suppressed in tomato plants damaged by *E.ludwigii*-inoculated larvae (Wand *et al.*, 2017). GOX is one of the principal proteins in *H.zea* saliva that mediates induced defenses in certain Solanaceae plants (Musser *et al.*, 2002; Tian *et al.*, 2012). GOX proteins on wounded sites of tomato leaves using a specific GOX antibody showed that inoculation of *E.ludwigii* affects the secretion of GOX from the saliva of *H.zea* larvae. Western blots showed that more GOX protein was secreted by *E.ludwigii*-inoculated *H.zea* than by  $MgCl_2$  solution inoculated larvae. When the spinnerets of caterpillars were ablated to examine the effects of saliva on the manipulation of tomato induced defenses, tomato plants damaged by ablation of the spinneret larvae significantly reduced PPO activities compared with plants damaged by *E.ludwigii*-inoculated larvae with intact spinnerets. Higher PPO activity was observed in tomato plants treated with saliva collected from *E.ludwigii* inoculated larvae than that in plants treated with saliva collected from  $MgCl_2$  solution-inoculated caterpillars (Wand *et al.*, 2017).

PPO activity was significantly suppressed by 20 $\mu$ l of  $10^9$  CFU/ml of *E. ludwigii*. The expression levels of JA-responsive *Pin2* is suppressed and SA-responsive *Pr1a* (P4) is induced in tomato plants when treated with 20 $\mu$ l of *E. ludwigii* ( $10^9$  CFU/ml). This showed that the suppression of plant defenses by *E. ludwigii* is dose-dependent when *E. ludwigii* is directly applied to wounded tomato plants (Wand *et al.*, 2017). GOX is the most abundant protein identified in *H.zea* saliva and plays a key role in the induction of defenses in tomato (Tian *et al.*, 2012). GOX from the *H.zea* larvae catalyzes the conversion of glucose to gluconic acid and hydrogen peroxide ( $H_2O_2$ ) (Kwakman *et al.*, 2010).  $H_2O_2$  could also induce defense genes and this is the likely factor in inducing plant defenses (Orozco-Cardenas *et al.*, 2001). GOX that play an important role in triggering JA-signaling responsive defenses in tomato plants. The function of GOX in the activation of plant defenses is planted species-dependent. For example, saliva or GOX from the labial glands of *H.zea* served as an effector to suppress induced defenses of *Nicotiana tabacum* (Musser *et al.*, 2005) by contrast, tomato JA-signaling regulated defense responses were triggered by GOX from *H.zea* larvae (Tian *et al.*, 2012).

The sap-feeding plant pest aphids harbor the endosymbiont *Buchnera aphidicola*, which is essential for their fecundity and survival. During plant penetration and feeding, aphids secrete saliva that contains proteins predicted to alter plant defenses and metabolism. GroEL is one of the abundant proteins in bacteria and has been shown to elicit immune responses in animal systems and GroEL induces enhanced resistance to aphids (Chaudhary *et al.*, 2014).

### Herbivore Associated-Microbe Suppresses Induced Plant Defenses

*Hamiltonella defensa* infection benefits whiteflies by suppressing induced plant defenses in tomato. *H. defensa* is confined within specialized insect cells called bacteriocytes in *B. tabaci*, it is not likely to be injected directly into the plant

via the stylets during feeding (Gottlieb *et al.*, 2008). *Hamiltonella defensa* infection improves *B. tabaci* performance on tomato by which seven days after releasing newly emerged adult whiteflies, *B. tabaci* infected with *H. defensa* performed significantly better on tomato compared to uninfected controls and the adult whitefly survival was 20% higher (Su *et al.*, 2015). The activity of PPO and POD was assessed in tomato plants after 24-h and 48-h period of infestation with H- or H+ whiteflies, in which PPO and POD responses in plants were significantly higher in H- than in H+ or control treatments. Compared with feeding by H- whiteflies, feeding by H+ whiteflies decreased the expression of the JA-responsive *LOX*, *AOS*, and *Chi9* but increased the expression of the SA-responsive *PR-1(P4)* in tomato plants. These results indicate that the presence of *H. defensa* in whiteflies suppresses anti-herbivore defenses (Su *et al.*, 2015). The whitefly saliva contains elicitors attributed to infection with *H. defensa*, by which the saliva from H- or H+ whiteflies to mechanically damaged plants and measured how saliva affects the expression of induced defense genes. The relative expression levels of defense marker genes were evaluated by quantitative RT-PCR. Expression levels of the JA responsive genes *LOX*, *AOS*, and *Chi9* were significantly lower in plants exposed to saliva from H+ relative to H- whiteflies, whereas the opposite was true for the expression of the SA-responsive *PR-1(P4)*. Symbiont-mediated suppression of plant defenses potentially involves antagonism between the JA and SA signaling pathways (Su *et al.*, 2015).

Gut-associated bacteria of lepidopterans can promote plant defense, produce digestive proteases, metabolize toxic plant compounds and appear to help in the hydrolysis or synthesis of N-acyl amino acid conjugates, which are elicitors of JA-mediated plant defenses (Ping *et al.*, 2007). FAW gut-associated microbes regulate herbivore-induced defenses and enhance the insect performance on tomato plants. *Pantoea ananatis* and Enterobacteriaceae-1 (*Serratia / Rahnella*) were Jasmonic acid defense-suppressing bacteria in FAW oral secretions and these bacteria did not alter salivary protein abundance or the activity of insect salivary enzymes. They directly regulate plant defenses upon their secretion through the insect regurgitant. The bacteria themselves or bacteria-derived components are eliciting specific plant defensive pathways. The effect of FAW-associated bacteria on JA-related plant defense responses appears to be host plant-specific. This could be due to differences in signal transduction pathways or receptor-mediated recognition of different host plants (Acevedo *et al.*, 2016).

### Herbivore Secreted Microbe Induce Defense Responses in Preferred and Non-Preferred Host Plants

False potato beetle (*Leptinotarsa decemlineata*) has been reported as a specialist on solanaceous weeds such as horsenettle, but also feed on other solanaceous plants such as ground cherry, husk tomato, and nightshade (Jr and Fasulo, 2015). Polyphenol oxidase is one of the JA-regulated enzymes that serves an anti-nutritive role by disrupting the normal digestive processes of herbivores (Bosch *et al.*, 2014). Both wounded tomato and horsenettle plants treated with oral secretion (OS) collected from untreated larvae showed lower PPO activities than those treated with water or OS from AB (antibiotic) treated larvae. These results were similar to plants damaged by AB or non-AB treated larvae. This shows that the microbes in OS from FPB larvae suppressed both the preferred and non-preferred plant induced defenses (Wang *et al.*, 2016).

The mechanism of the suppression in plant defenses caused by bacterial isolates was quantified the expression levels of JA- and SA-regulated genes in both tomato and horsenettle leaves. Both the plants were wounded and treated with *Pantoea sp.*, which suppressed PPO activities in both tomato and horsenettle, but *Enterobacter sp.* decreased PPO activities only in tomato. This shows that both *Pantoea sp.* and *Enterobacter sp.* decreased JA-responsive *CysPI* expression alongside increasing SA-regulated *PrI* expression and results that the different microbes in insects have species-specific

effects on different host-plants (Wang *et al.*, 2016).

## TOOLS TO STUDY THE MICROBIOLOGY OF PLANT-ASSOCIATED INSECTS

- Phylogenetic position of the bacterium can be determined from sequencing methods. (Parallel sequencing of 16S rRNA gene) (Douglas, 2013).
- Genome-based methods, such as metabolic modeling, can assist in identifying specific nutritional requirements and have been used successfully to construct suitable culture media (Douglas, 2013).

## CONCLUSIONS

More studies are needed to determine the mechanisms by which Herbivore-associated bacteria regulate JA-inducible plant defenses (Frago *et al.*, 2012). Further studies to achieve selective elimination of single bacterial taxa, to pinpoint which bacteria are responsible for manipulating plant defense (Schausberger, 2018). Physiological and genetic studies are needed to directly demonstrate the role of microbes in assisting detoxification in insect gut (Sikano *et al.*, 2016). Future studies are required to unravel why and how the microbes alter their host's behavior (Zhu *et al.*, 2014). Whether the effects of microbial symbionts on herbivore physiology affect plant responses to herbivory remains to be investigated (Zhu *et al.*, 2014).

## REFERENCES

1. Acevedo, F. E., M. Peiffer, C. W. Tan, A. G. Jones, K. Hoover, B. A. Stanley, A. Stanley, J. Wang, C. Rosa, D. Luthe and G. Felton. 2016. Fall armyworm-associated gut bacteria modulate plant defense responses. **Molecular Plant-Microbe Interactions**, doi: 10.1094/MPMI-11-16-0240-R.
2. Arimura, G.I., K. Matsui and J. Takabayashi. 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. **Plant Cell Physiol.**, 50: 911-23; <http://dx.doi.org/10.1093/pcp/pcp030>.
3. Bhonwong, A., M. J. Stout, J. Attajarusit and P. Tantasawat. 2009. Defensive role of tomato polyphenol oxidases against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). **J. Chem. Ecol.**, 35: 28-38; <http://dx.doi.org/10.1007/s10886-008-9571-7>.
4. Bonaventure, G. 2012. Perception of insect feeding by plants. **Plant Biology**, 14: 872– 880.
5. Boone, C. K., K. K. Ring, A. C. Mapes, A. S. Adams, J. Bohlmann and K. F. Raffa. 2013. Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. **Chem. Ecol.**, 39: 1003–1006; doi: 10.1007/s10886-013-0313-0.
6. Bosch, M., S. Berger, A. Schaller and A. Stintzi. 2014. Jasmonate-dependent induction of polyphenol oxidase activity in tomato foliage is important for defense against *Spodoptera exigua* but not against *Manduca sexta*. **BMC Plant Biol.**, 14: 257.
7. Casteel, C. L., A. K. Hansen, L. L. Walling and T. D. Paine. 2012. Manipulation of plant defense responses by the tomato psyllid (*Bactericera cockerelli*) and its associated endosymbiont *Candidatus Liberibacter Psyllae*. **PLoS ONE**, 7(4): doi: 10.1371/journal.pone.0035191.
8. Chaudhary, R., H. S. Atamiana, Z. Shenc, S. P. Briggsc and I. Kaloshiana. 2014. GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense. **Microbiology**, 11(24): 8919–8924.
9. Chung, S. H., C. Rosa, E. D. Scully, M. Peiffera, J. F. Tookera, K. Hoovera, D. S. Luthe and G. W. Feltona. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. **Ecology**, doi: 10.1073/pnas.1308867110.

10. Douglas, A. E. 2009. The microbial dimension in insect nutritional ecology. **Functional Ecology**, 23: 38-47; doi: 10.1111/j.1365-2435.2008.01442.x.
11. Douglas, A. E. 2013. Microbial brokers of insect-plant interactions revisited. **Chem. Ecol.**, 39:952–961; doi: 10.1007/s10886-013-0308-x.
12. Dudareva, N., F. Negre, D. A. Nagegowda and I. Orlova. 2006. Plant volatiles: recent advances and future perspectives. **Crit. Rev. Plant Sci.**, 25: 417-40; <http://dx.doi.org/10.1080/07352680600899973>.
13. Erb, M., S. Meldau and G. A. Howe. 2012. Role of phytohormones in insect-specific plant reactions. **Trends in Plant Science**, 17: 250–259.
14. Mohamed, E. A. M., Dessoky, E. S., Attia, A. A., & Hassan, M. M. (2014). Evaluation of genetic fidelity of in vitro raised plants of the important medicinal plant Harmal (*Rhazya stricta* Decne) using RAPD and ISSR markers. *International Journal of agricultural Science and Research*, 4(3), 115-124.
15. Frago, E., M. Dicke and H. C. J. Godfray. 2012. Insect symbionts as hidden players in insect plant interactions. **Trends in Ecology and Evolution**, 27(12); doi: [org/10.1016/j.tree.2012.08.013](http://dx.doi.org/10.1016/j.tree.2012.08.013).
16. Ghanim, M. and S. Kontsedalov. 2009. Susceptibility to insecticides in the Qbiotype of *Bemisia tabaci* is correlated with bacterial symbiont densities. **Society of Chemical Industry**, 65:939–942; doi: 10.1002/ps.1795.
17. Giron, D., W. Kaiser, N. Imbault and J. Casas. 2007. Cytokinin-mediated leaf manipulation by a leafminer caterpillar. **Physiology**, 3:340-343; doi:10.1098/rsbl.2007.0051.
18. Gottlieb, Y., M. Ghanim, G. Gueguen, S. Kontsedalov, F. Vavre and F. Fleury. 2008. Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. **FASEB Journal**, 22: 2591– 2599.
19. Hanley, M. E., B. B. Lamont, M. M. Fairbanks and C. M. Rafferty. 2007. Plant structural traits and their role in anti-herbivore defense. *Perspec. Plant Ecol. Evol. Syst.*, 8: 157-78; <http://dx.doi.org/10.1016/j.ppees.2007.01.001>.
20. Hansen, A. K. and N. A. Moran. 2013. The impact of microbial symbionts on host plant utilization by herbivorous insects. **Molecular Ecology**, doi: 10.1111/mec.12421.
21. Hoover, K., M. Grove, M. Gardner, D. P. Hughes, J. Neil and J. Slavicek. 2011. A Gene for an extended phenotype. **Science**, 333; doi: 10.1126/science.1209199
22. Howe, G.A. and G. Jander. 2008. Plant immunity to insect herbivores. **Annu. Rev. Plant Biol**, 59: 41-66; <http://dx.doi.org/10.1146/annurev. arplant.59.032607.092825>.
23. Hughes, D. P., J. Brodeur and F. Thomas. 2012. Host manipulation by parasites. Oxford, UK: Oxford University Press.
24. Jr, R. L. J. and T. R. Fasulo. 2015. Colorado potato beetle, *Leptinotarsa decemlineata* (say), and false potato beetle, *Leptinotarsa juncta* (Germar) (Insecta: Coleoptera: Chrysomelidae). EDIS website. <http://edis.ifas.ufl.edu/in303>. Accessed November 2015.
25. Kwakman, P. H. S., A. A. te Velde, L. de Boer, D. Speijer, C. M. J. E. Vandenbroucke-Grauls and S. A. J. Zaat. 2010. How honey kills bacteria. **FASEB Journal**, 24: 2576– 2582.
26. Meyer, J. R. 2006. **Insect Herbivores**. Raleigh, North Carolina: NC State University.
27. Orozco-Cardenas, M. L., J. Narvaez-Vasquez and C. A. Ryan. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. **Plant Cell**, 13: 179– 191.



28. Pieterse, C. M. J. and L. C. Van Loon. 2004. NPR1: the spider in the web of induced resistance signaling pathways. **Curr. Opin. Plant Biol.**, 7: 456-64; <http://dx.doi.org/10.1016/j.pbi.2004.05.006>.
29. Ping, L., R. Böhler, A. Mithfer, A. Svatos, D. Spiteller, K. Dettner, S. Gmeiner, J. Piel, B. Schlott and W. Boland. 2007. A novel Dps-type protein from insect gut bacteria catalyses hydrolysis and synthesis of N-acyl amino acids. **Environ. Microbiol.**, 9: 1572-1583.
30. Onwuachu, U. I., Aboh, Uju. A., & Iwuoha, Godson. (2014). Extraction, characterization and workability of some local plants dyes as acid-base indicators. *International Journal of Research in Applied, Natural and Social Sciences*, 7, 1-6.
31. Rivas-San Vicente, M. and J. Plasencia. 2011. Salicylic acid beyond defence: its role in plant growth and development. **J. Exp. Bot.**, 62: 3321-38; <http://dx.doi.org/10.1093/jxb/err031>.
32. Schausberger, P. 2018. Herbivore-associated bacteria as potential mediators and modifiers of induced plant defense against spider mites and thrips. **Frontiers in Plant Science**, 9: doi:10.3389/fpls.2018.01107.
33. Schwartzberg, E. G. and J. H. Tumlinson. 2014. Aphid honeydew alters plant defence responses. **Functional Ecology**, 28: 386–394.
34. Shigenobu, S., H. Watanabe, M. Hattori, Y. Sakaki and H. Ishikawa. 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. **Nature**, 407: 81-86.
35. Shikano, I., C. Rosa, C. W. Tan and G. W. Felton. 2017. Tritrophic interactions: Microbe-mediated plant effects on insect herbivores. **Phytopathology**, 12: doi: org10.1146/annurev-phyto-080516-035319.
36. Shin, S. C., S. H. Kim, H. You, B. Kim, A. C. Kim, K. A. Lee, J. H. Yoon, J. H. Ryu. and W. J. Lee. 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling . **Science**, 334: 670-674.
37. Sloan, D. B. and N. A. Moran. 2012. Endosymbiotic bacteria as a source of carotenoids in whiteflies. **Evolutionary Biology**, 8: 986-989: doi:10.1098/rsbl.2012.0664.
38. Su, Q., K. M. Oliver, W. Xie, Q. Wu, S. Wang and Y. Zhang. 2015. The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defenses in tomato. **Functional Ecology**, 29: 1007–1018: doi:10.1111/1365-2435.12405.
39. Tian, D., M. Peiffer, E. Shoemaker, J. Tooker, E. Haubruge, F. Francis, D. S. Luthe and G. W. Felton. 2012. Salivary glucose oxidase from caterpillars mediates the induction of rapid and delayed-induced defenses in the tomato plant. **PLoS ONE**, 7: e36168.
40. Usha Rani, P. and Y. Jyothsna. 2010. Biochemical and enzymatic changes in rice as a mechanism of defense. **Acta Physiol. Plant**, 32: 695-701; <http://dx.doi.org/10.1007/s11738-009-0449-2>.
41. Wang, J., M. Peiffer, K. Hoover, C. Rosa, R. Zeng and G. W. Felton. 2017. *Helicoverpa zea* gut-associated bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s). **New Phytologist**, doi: 10.1111/nph.14429.
42. Wang, J., S. H. Chung, M. Peiffer, C. Rosa, K. Hoover, R. Zeng and G. W. Felton. 2016. Herbivore oral secreted bacteria trigger distinct defense responses in preferred and non-preferred host plants. **Chem. Ecol.**, doi: 10.1007/s10886-016-0712-0.
43. War, A. R., M. G. Paulraj, M. Y. War and S. Ignacimuthu. 2011. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). **Plant Signal Behav.**, 6: 1787-92; <http://dx.doi.org/10.4161/psb.6.11.17685>.
44. War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu and H. C. Sharma. 2012. Mechanisms of plant defense against insect herbivores. **Plant Signaling & Behavior**, 7(10):1306-1320.

45. Wu, D., S. C. Daugherty, S. E. V. Aken, G. H. Pai, K. L. Watkins, H. Khouri, L. J. Tallon, J. M. Zaborsky, H. E. Dunbar, P. L. Tran, N. A. Moran and J. A. Eisen. 2006. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. **Plos Biology**, 4(6): doi:10.1371/journal.pbio.0040188.
46. Gupta, A., Naraniwal, M., & Kothari, V. (2012). Modern extraction methods for preparation of bioactive plant extracts. *International journal of applied and natural sciences*, 1(1), 8-26.
47. Zhao, L. Y., J. L. Chen, D. F. Cheng, J. R. Sun, Y. Liu and Z. Tian. 2009. Biochemical and molecular characterizations of *Sitobion avenae*-induced wheat defense responses. **Crop Prot.**, 28: 435-42; <http://dx.doi.org/10.1016/j.cropro.2009.01.005>.
48. Zhu, F., E. H. Poelman and M. Dicke. 2014. Insect herbivore-associated organisms affect plant responses to herbivory. **New Phytologist**, 204: 315–321 doi: 10.1111/nph.12886.